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Persistent organic pollutants and penile bone mineral density in East Greenland and Canadian polar bears (*Ursus maritimus*) during 1996–2015

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ABSTRACT

Persistent organic pollutants (POPs) are long-range transported to the Arctic via atmospheric and oceanic currents, where they biomagnify to high concentrations in the tissues of apex predators such as polar bears (*Ursus maritimus*). A major concern of POP exposure is their physiological effects on vital organ-tissues posing a threat to the health and survival of polar bears. Here we examined the relationship between selected POPs and baculum bone mineral density (BMD) in the East Greenland and seven Canadian subpopulations of polar bears. BMD was examined in 471 bacula collected between years 1996–2015 while POP concentrations in adipose tissue were determined in 67–192 of these individuals collected from 1999 to -2015. A geographical comparison showed that baculum BMD was significantly lowest in polar bears from East Greenland (EG) when compared to Gulf of Boothia (GB), Southern Hudson (SH) and Western Hudson (WH) Bay subpopulations (all $p < 0.05$). The calculation of a T-score osteoporosis index for the EG subpopulation using WH bears as a reference group gave a T-score of -1.44 which indicate risk of osteopenia. Concentrations of ΣPCB_{74} (polychlorinated biphenyls), ΣDDT_3 (dichlorodiphenyltrichloroethanes), *p,p′*-DDE (dichlorodiphenyldichloroethylene), ΣHCH_3 (hexachlorohexane) and α-HCH was significantly highest in EG bears while ΣPBDE (polybrominated diphenyl ethers), BDE-47 and BDE-153 was significantly highest in SH bears (all $p < 0.04$). Statistical analyses of individual baculum BMD vs. POP concentrations showed that BMD was positively correlated with ΣPCB_{74}, CB-153, HCB (hexachlorobenzene), ΣHCH, β-HCH, ClBz (chlorobenzene), ΣPBDE and BDE-153 (all $p < 0.03$). In conclusion, baculum density was significantly lowest in East Greenland polar bears despite the positive statistical correlations of BMD vs. POPs. Other important factors such as nutritional status, body mass and body condition was not available for the statistical modelling. Since on-going environmental changes are known to affect these, future studies need to incorporate nutritional, endocrine and genetic parameters to further understand how POP exposure may disrupt bone homeostasis and affect baculum BMD across polar bear subpopulations.

1. Introduction

Climatic changes as well as infectious diseases and persistent organic pollutants (POPs) are considered the most substantial environmental stressors of the Arctic ecosystem (AMAP, 2015; Jenssen et al., 2015; Letcher et al., 2010; Sonne et al., 2012). The presence of POPs in the Arctic marine environment is the result of long-range atmospheric and oceanic transport, which has occurred since the 1940s from lower latitude sources in the industrialized parts of the world (AMAP, 1998, 2004). Due to the lipophilic nature of many POPs, these chemicals persist in the slow-growing and lipid-rich Arctic food chains (Letcher et al., 2010). Consequently, high POP concentrations are found in the

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Inuit populations and in marine top predators that consume large amounts of high trophic level marine mammals with East Greenland and Hudson Bay as particular hotspots (AMAP, 2015; Dietz et al., 2013a, 2013b; Letcher et al., 2010, 2018; McKinney et al., 2013).

Recently, polar bears have received considerable attention as a vulnerable Arctic species that is highly influenced by climatic change (Jenssen et al., 2015; Wiig et al., 2015). Thus, it is argued that the projected sea ice loss across the Arctic Ocean will restrict polar bears' access to principal prey such as ringed seals (*Phoca hispida*) during autumn (Durner et al., 2009; Molnár et al., 2011; Stirling and Derocher, 2012). In addition, polar bears are top predator species and therefore at greater risk of severe population declines due to POP exposure (Jenssen et al., 2015; Letcher et al., 2010; Sonne, 2010). The East Greenland ecosystem carry the highest loads of POPs in the Arctic and therefore polar bears from these subpopulations are among the most contaminated (Letcher et al., 2010). Increases in biomagnification of POPs has occurred in East Greenland polar bears over the last decades mainly because changes in ice dynamics that has led to dietary changes and toward more highly polluted prey i.e. hooded (*Cystophora cristata*) and harp (*Pagophilus groenlandicus*) seals (McKinney et al., 2013). The consequence of these dietary changes and elevated POP exposure is an increase in the risk for effects on growth and development of, for example, reproductive organs and the immune and skeletal system (Desforges et al., 2016; Dietz et al., 2015; Letcher et al., 2010; Sonne, 2010; Sonne et al., 2006, 2012).

Bone formation and resorption is controlled by multiple physiological factors such as hormones, vitamins and micronutrients (Barret et al., 2010; Herlin et al., 2010). POPs are known to disrupt bone homeostasis (Lind et al., 2003, 2004) and in polar bears there has previously been reported inverse relationships between bone density and several POP compounds (Sonne, 2010; Sonne et al., 2004, 2006). A study by Sonne et al. (2015) even suggested that changes in the baculum density of East Greenland polar bears may lead to population declines as reduced strength could lead to fractures and inability to successfully mate.

Canadian polar bear subpopulations are, with the exception of Hudson Bay, less contaminated with POPs compared the East Greenland subpopulation (Norstrom et al., 1998; McKinney et al., 2011; Verreault et al., 2005). A comparative study of baculum bone density and POPs in East Greenland and Canadian polar bears is therefore warranted (Sonne et al., 2015). The aim of the present study was to investigate possible geographical differences in baculum BMD, as well as correlations between POP concentrations and baculum BMD.

2. Materials and methods

2.1. The sample

BMD was measured in 471 bacula collected in the period 1996–2015. These were from eight management areas including East Greenland (EG) ($n = 137$), Baffin Bay (BB) ($n = 32$), Davis Strait (DS) ($n = 28$), Lancaster Sound (LS) ($n = 62$), Gulf of Boothia (GB) ($n = 31$), Foxe Basin (FB) ($n = 80$), Southern Hudson Bay (SH) ($n = 74$) and Western Hudson Bay (WH) ($n = 27$) (Fig. 1). All bacula were collected as part of research projects based on local Inuit subsistence hunting (Letcher et al., 2010, 2018; Sonne, 2010; Sonne et al., 2012, 2015). The bacula were stored frozen at −20 °C and manually cleaned, macerated and dried at room temperature prior to analyses. POP concentrations was available from SH ($n = 72$–74), WH (n = 27) and EG ($n = 15$–91) as part of previous studies conducted 1999–2015 (Dietz et al., 2013a, 2013b, Unpubl. data; Letcher et al., 2010, 2018; McKinney et al., 2010). The individual age estimations was performed by counting the cementum growth layer groups (GLG) of the lower right incisor (I_3) after decalcification, thin sectioning (14 µm) and staining with toluidine blue as described by Hensel and Sorensen (1980) and Dietz et al. (1991).

2.2. Bone mineral density (BMD) measurements

X-Ray osteodensitometry was applied to determine baculum bone mineral density. The X-ray bone densitometer (model XR 26; Norland Corporation, Fort Atkinson, WI, USA) determined BMD (calcium phosphate, hydroxyapatite; g/cm^2) using dual X-ray absorptiometry (DXA). The bacula were scanned in "research" mode (speed, 60 mm/s; resolution, 3.0 × 3.0 mm; width, 24.9 cm) and analysed using XR software (revision 2.4; Norland Corporation), which generated a picture of the bone segment and calculated the BMD of hydroxyapatite in grams per square centimetre. The DXA scanner was calibrated daily using a phantom with known bone mineral density. In addition, the precision was tested by a 10 × rescanning (mean ± SD, 521.96 ± 0.60 g/cm^2), which gave a precision of 99.88% ([1 – (SD/mean) × 100%]). T-score, a measure used in human medicine for calculating whether patients are in risk of osteoporosis (WHO, 2007) was estimated for East Greenland polar bears according to Sonne (2010): $\text{T-score}_{EG} = M_{EG}\text{-}M_{WH}/SD_{WH}$. M = mean, SD = standard deviation, EG = East Greenland polar bears, WH = Western Hudson polars used as reference group holding the highest BMD values. Normal bone: T-score > −1; Osteopenia: −2.5 < T-score < −1; Osteoporosis: T-score < −2.5.

2.3. Persistent organic pollutant (POP) determination

The determination of selected POPs in adipose tissue was conducted at the Organic Contaminant Research Laboratory at Environment and Climate Change Canada's National Wildlife Research Centre (Carleton University) in Ottawa, Canada. Contaminants were extracted from the tissue and determined by gas chromatography-single quadrupole mass spectrometry (GC–MS) as described in McKinney et al. (2010). As part of an on-going AMAP (Arctic Monitoring and Assessment Program) programme; all adipose tissue samples were analysed by the same laboratory for 74 polychlorinated biphenyl congeners (ΣPCB_{74}) including CB-18, −17, −16/32, −31, −28, −33/20, −22, −52, −49, −47/48, −44, −42/59, −64/41, −74, −70/76, −66, −56/60, −95, −92, −101/90, −99, −97, −87, −85, −110, −118, −114, −105, −151, −149, −146, −153, −141, −130, −137, −138, −158, −128, −167, −156, −157, −179, −176, −178, −187/182, −183, −174, −177, −171, −172, −180, −170/190, −189, −202, −200, −199, −196/203, −208, −195, −207, −194, −206 and −201, dichlorodiphenyltrichloroethanes (ΣDDT) including *p,p'*-DDE (dichlorodiphenyldichloroethylene), *p,p'*-DDD (dichlorodiphenyldichloroethane) and *p,p'*-DDT, hexachlorocyclohexanes (ΣHCH) including α-, β- and γ-HCH, hexachlorobenzene (HCB), chlorobenzene (ΣClBz), chlordanes including oxychlordane, *trans*-chlordane, nonachlor III (MC6), *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide, 14 polybrominated diphenyl ethers ($\Sigma PBDE_{14}$) including the congeners BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −190, −209 and hexabromocyclododecane (HBCDD). The POP concentrations are expressed in lipid weight (lw). Quantification methods have been reported in detail by e.g. Letcher et al. (2018) and McKinney et al. (2010, 2011).

2.4. Statistical analyses

First, a regression analysis was conducted to investigate the relationship between age and BMD. To secure comparability among the polar bear subpopulations and to take sample size into account, only individuals being 3–9 years of age were used to investigate the growth pattern and differences in BMD between subpopulations by analysis of covariance models (ANCOVA). The explanatory variables were age and age^2 assuming that growth followed a polynomial curve. A test of least square means (LSM) of significant factors was applied for pairwise comparisons in BMD among polar bear subpopulations. LSM (or marginal means) are estimates of the means when controlled for co-variables (age). Analyses of covariance (ANCOVA) with Tukey's post hoc was used to analyse differences in ΣPCB concentrations among EG, SH and WH subpopulations taking age into consideration. To obtain

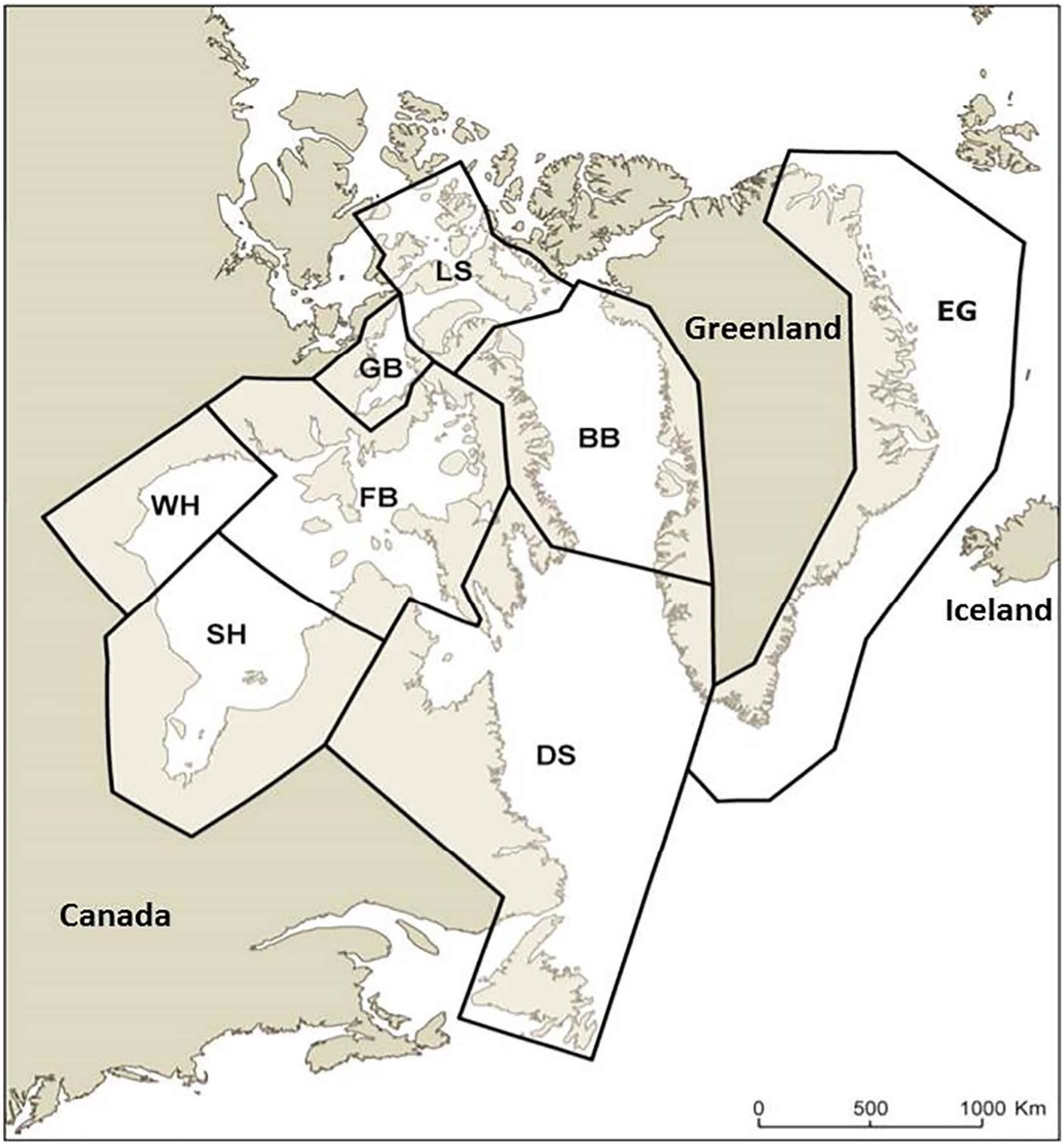

Fig. 1. Map of Canadian and East Greenland polar bear subpopulations. WH: Western Hudson Bay; SH: Southern Hudson Bay; FB: Foxe Basin; GB: Gulf of Boothia; LS: Lancaster Sound; BB: Baffin Bay, DS: Davis Strait; EG: East Greenland. Modified from Sonne et al. (2015).

sufficient statistical power, the relationship of BMD vs. POPs was evaluated using regression analyses on all pooled bears available from East Greenland, Southern Hudson and Western Hudson Bay aged 1–29 years. Age and age^2 together with logarithmic transformed POP concentrations composed the explanatory variables. All statistical analyses were performed using the free software R version 3.01 (R Development Core Team, 2013). The level of significance was set to $\alpha = 0.05$.

3. Results

3.1. BMD in subpopulations

Overall, BMD increased significantly with age in all subpopulations following a polynomial growth curve (all $p < 0.001$). To test for geographical differences in BMD among the eight subpopulations, data from a subsample of 349 individuals all aged 3–9 years was used. The basic statistics for this subsample are shown in Table 1. East Greenland polar bears had the lowest baculum BMD values while values from the SH and WH were among the highest (Table 1, Fig. 2). A pairwise test of the LSM values showed that significant differences were found between GB, SH and WH and all of these being significantly higher than in polar bears from EG (all $p < 0.05$, Tables 1, 2). In addition, non-significant trends showed that polar bears from WH had higher baculum BMD compared to polar bears from BB, and that polar bears from DS and FB had higher baculum BMD compared to polar bears from EG (all $p \leq 0.07$).

3.2. BMD and POPs

To test for the relationship between BMD and POPs and to increase the statistical power, pooled data from a subsample of the three

Table 1
Basic statistics for age and baculum BMD using a subsample of 349 polar bears aged 3–9 years from each of the eight subpopulations during 1996–2015 to test for geographical differences. EG: East Greenland; BB: Baffin Bay; DS: Davis Strait; LS: Lancaster Sound; GB: Gulf of Boothia; FB: Foxe Basin; SH: Southern Hudson Bay; WH: Western Hudson Bay.

	EG	BB	DS	LS	GB	FB	SH	WH
	n = 56	n = 32	n = 28	n = 62	n = 31	n = 80	n = 40	n = 20
Age (years)								
Mean ± SD	5.36 ± 1.74	4.91 ± 1.84	5.39 ± 1.87	4.77 ± 1.62	4.74 ± 1.88	4.55 ± 1.58	5.59 ± 1.45	4.95 ± 2.11
Min-Max	3–9	3–9	3–9	3–9	3–9	3–9	3–9	3–9
BMD (g/cm2)								
LS Mean ± SE[a]	0.26 ± 0.007	0.27 ± 0.007	0.29 ± 0.008	0.28 ± 0.006	0.29 ± 0.008	0.28 ± 0.005	0.29 ± 0.009	0.31 ± 0.01
Min-Max	0.13–0.42	0.16–0.38	0.17–0.44	0.16–0.41	0.17–0.42	0.15–0.45	0.21–0.41	0.20–0.42

[a] LS Mean was significantly lower in EG when compared to GB, SH and WH (see Table 2).

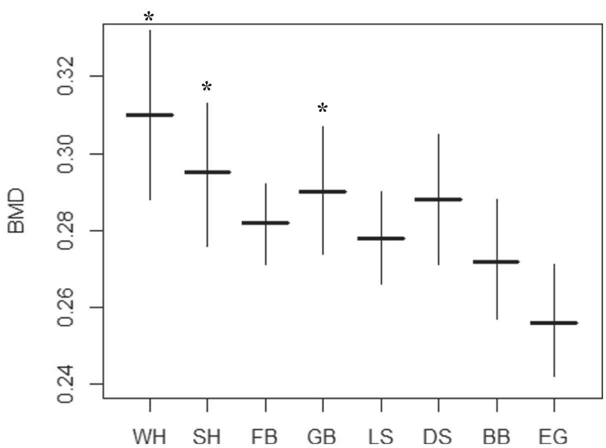

Fig. 2. Baculum bone mineral density (BMD) index as least square means (LSM ± SE) for the eight polar bear subpopulations based on 3–9 year old bears collected in the period 1996–2015. *: EG significantly lower compared to WH, SH and GB (all $p < 0.03$).

Table 2
p-values and t-ratios from the LSM ANCOVA analyses comparing baculum BMD showing that BMD is significantly higher in WH, SH and GB when compared to the EG polar bear subpopulations. A positive t-ratio indicates that the subpopulation first mentioned has higher BMD value.

Populations	p	t ratio
GB-EG	0.028	3.25
SH-EG	0.024	3.31
WH-EG	< 0.001	4.21

subpopulations SH (n = 72–74), WH (n = 27) and EG (n = 15–91) all aged 1–29 was used (Tables 3, 4). Overall, ΣPCB_{74} was the group of contaminants found in the highest concentrations. When comparing the three subpopulations, statistical analyses showed that concentrations of ΣPCB_{74}, ΣDDT, *p,p* ′-DDE, ΣHCH and α-HCH was significantly highest in EG polar while ΣPBDE (polybrominated diphenyl ethers), BDE-47 and BDE-153 was significantly highest in SH bears (all $p < 0.04$) (Table 3, Fig. 3). ΣPCB_{74} showed clear significant positive correlation with baculum BMD ($p < 0.001$) (Fig. 4). Likewise, significant positive correlations were also found between BMD and CB-153, HCB, ΣHCH, β-HCH, ClBz, ΣPBDE and BDE-153 (all $p < 0.03$, Table 4).

3.3. T-score

Bears from the WH subpopulation was used as a reference group to the EG bears having high POP concentrations and low BMD levels. The calculation of a T-score osteoporosis index for the EG subpopulation gave a T-score$_{EG}$ = 0.307–0.360/0.037 = −1.44 indicating risk of osteopenia. In the overall collection of 471 bacula, only a single healed fracture was found being an adult male from the Davis Strait (Fig. 5). The BMD of this adult male was 0.41 g/cm^2 and it had a T-score = 0.41–0.360/0.037 = 1.35, which indicate that this particular adult male was not in risk of having osteoporosis related fractures. No POP data were available for this specific adult male. It is however known that Davis Strait polar bears are known to be among the subpopulation of Canadian polar bears with the lowest POP levels and it is therefore likely that this specimen has been exposed to low concentrations of e.g. PCBs.

4. Discussion

4.1. Subpopulations and T-scores

Bone mineral composition in mammals is based on a complex set of interrelated mechanisms (Barret et al., 2010) making disturbances in the homeostasis very challenging to investigate and comprehend. POPs have previously been shown to affect bone tissue in various wildlife species and by various modes of action (Lind et al., 2004; Sonne, 2010; Sonne et al., 2015). Sonne et al. (2015) showed that baculum BMD was lower in EG polar bears compared to Canadian polar bears and suggested that this could be due to higher PCB concentrations in EG. East Greenland polar bears are considered the most POP contaminated subpopulation (McKinney et al., 2010, 2011; Norstrom et al., 1998; Verreault et al., 2005) and had in the present study the lowest baculum BMD and highest PCB concentrations. The baculum T-score estimation showed that the EG subpopulation is in risk of having osteopenia when compared to WH (Sonne, 2010) and that has been showed for skull BMD in East Greenland polar bear males as well (Daugaard-Petersen et al., 2018). A reduction of polar bear baculum BMD could lead to fractures and influence the bears' fertility (Sonne et al., 2015). Only a single polar bear from DS had a baculum with healed fracture was seen. That adult bear had a high BMD, a high T-score and a low estimated POP exposure. It is therefore unlikely that the fracture is caused by exposure to e.g. PCBs known to cause osteoporosis via endocrine disruption (Sonne, 2010). The fact that we only found a single baculum with healed fracture could be due to the "healthy worker effect" where only healthy bears are taken as part of the subsistence hunt (Sonne, 2010).

Finding geographical differences in morphology among polar bears is not surprising since polar bears in general exhibit considerable regional variations in growth rate and body size (Kurtén, 1964; Manning, 1971; Wilson, 1976; Atkinson et al., 1996; Derocher and Stirling 1998; Stirling and Lunn, 1998; Derocher and Wiig, 2002). The reason for this may be environmental factors as well as different genetic pools in the various subpopulations (Dyck et al., 2004; Paetkau et al., 1999; Sonne et al., 2007).

4.2. BMD vs. POPs

We found positive correlations between BMD and ΣPCB, CB-153, HCB, ΣHCH, ClBz, ΣPBDE and BDE-153 concentration based on a

Table 3
Basic statistics (Mean ± SD, Min-Max, n) for age, baculum BMD and contaminant concentrations in East Greenland (EG), Southern Hudson (SH) and Western Hudson (WH) polar bears using a subsample of 67–192 bears aged 1–29 years during 1999–2015. -: data not available. POP data are given in ng/g lw.

	EG	SH	WH
Age (years)	6.9 ± 5.4 (1–28, 91)	9.3 ± 6 (2–27, 74)	11 ± 9.6 (3–29, 27)
BMD (g/cm^2)	0.3 ± 0.1 (0.1–0.5, 91)	0.32 ± 0.07 (0.17–0.46, 74)	0.31 ± 0.07 (0.2–0.4, 27)
ΣPCB$_{74}$	10,598 ± 7350 (2608–52,653, 91)[a]	6348 ± 4436 (0–29,881, 74)	5250 ± 3201 (1486–16,747, 27)
CB-153	3405 ± 1989 (885–12,104, 91)	2648 ± 1970 (663–15,188, 74)	2224 ± 1440 (620–7718, 27)
ΣCHL	1333 ± 901 (403–3720, 15)	1623 ± 1016 (0–5471, 74)	2339 ± 1031 (647–4659, 27)
ΣDDT$_3$	348 ± 293 (18–2044, 91)[b]	181 ± 136 (0–1096, 74)	188 ± 173 (36–611, 27)
p,p′-DDE	304 ± 254 (15–1695, 91)[b]	173 ± 127 (47–1026, 74)	176 ± 161 (32–565, 27)
HCB	189 ± 249 (21–1561, 67)	–	–
ΣHCH$_3$	186 ± 140 (11–1203, 91)[b]	237 ± 93 (0–454, 74)	294 ± 100 (0–575, 27)
α-HCH	30 ± 32 (0–219, 91)[b]	62 ± 31 (0–150, 74)	55 ± 18 (17–109, 27)
β-HCH	156 ± 121 (0–984, 91)	181 ± 71 (0–379, 72)	250 ± 92 (0–509, 27)
ClBz	256 ± 314 (32–1670, 47)	181 ± 68 (0–399, 74)	900 ± 1209 (81–4498, 27)
ΣPBDE$_{14}$	55 ± 33 (12–277, 91)	96 ± 51 (0–371, 74)[a]	51 ± 23 (21–120, 27)
BDE-47	32 ± 23 (0–138, 91)	43 ± 21 (9–110, 74)[a]	20 ± 8 (6–36, 27)
BDE-153	34 ± 52 (0–241, 29)	55 ± 55 (0–291, 74)[a]	32 ± 36 (1–149, 27)
HBCDD	29 ± 21 (4–138, 49)[c]	5.9 ± 6.8 (0–34, 74)	1.7 ± 4.1 (0–19, 27)

[a] Significantly highest ($p < 0.05$).
[b] Significantly highest ($p < 0.01$).
[c] Significantly highest ($p < 0.001$).

pooled material of SH, WH and EG subpopulations. Previous studies of EG polar bears have shown both negative and positive correlations between POPs and BMD in skulls and baculum (Daugaard-Petersen et al., 2018; Sonne et al., 2004, 2006, 2013). A reason for this could be that environmental changes has caused fluctuations in hormone, vitamin, fatty acid and POP composition over the past two decades (Bechshøft et al. 2016; Jenssen et al., 2015; McKinney et al., 2013). Since all these factors are known to affect BMD and the ratio of trabecular:cortical bone, it has likely biased our study (Johansson et al., 2002; Lind et al., 2000; Sonne et al., 2008). Unfortunately, data on endocrine and nutritional status was not available for the majority of the polar bears and therefore we could not parameterize and control for these in our statistical models.

Other studies of wildlife including grey seals (*Halichoerus grypus*) and alligators (*Alligator mississippiensis*) have reported on positive correlations between BMD (trabecular) and PCB concentrations (Lind et al., 2003, 2004). That BMD is increasing with increasing PCB concentrations does not necessarily mean better quality or stronger bone. Lind et al. (2000, 2004) showed that the dioxin-like CB-126 congener increased BMD of lumbar vertebrae in rats, which resulted in changes leading to an impaired bone quality. The impaired quality was seen as a decrease in collagen mass and bone bending strength, as well as an increase in cross-links in the bone's collagen molecules. It is difficult to conclude on bone strength and quality based on BMD alone and measures of bone fragility using e.g. p-QCT (peripheral Quantitative Computed Tomography) and biomechanical properties are necessary to further evaluate polar bear bone health (Lind et al., 2004, Sonne, 2010; Sonne et al., 2009b).

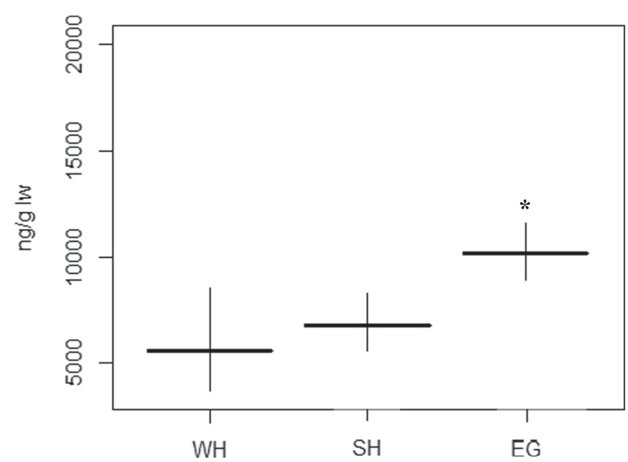

Fig. 3. ΣPCB$_{74}$ concentrations (least square mean and Min-Max) in the subcutaneous adipose tissue of polar bears from the subpopulations SH (n = 74), WH (n = 27) and EG (n = 91) collected in the period 1999–2015. *: EG significantly higher compared to WH and SH (both $p < 0.03$).

Table 4
Results from the multiple regression analyses of BacBMD (bone mineral density, g × cm^{-2}) = intercept + age + I (age^2) + log (POP) combining Table 3 data from WH, SH and EG polar bear subpopulations during 1999–2015. r^2: regression coefficient. p: p-value. F: F-value. n = sample size.

POPs	R^2	p (age)	p (age^2)	p (POP)	F (age)	F (age^2)	F (POP)	n
ΣPCB$_{74}$	0.59	< 0.001	< 0.001	< 0.01	105	53.0	15.2	192
CB-153	0.59	< 0.001	< 0.001	< 0.01	106	53.3	15.0	192
ΣCHL	0.42	< 0.001	< 0.001	0.76	24.7	11.6	0.09	116
ΣDDT$_3$	0.54	< 0.001	< 0.001	0.63	93.6	47.2	0.23	192
p,p′-DDE	0.54	< 0.001	< 0.001	0.53	94.5	47.6	0.40	192
HCB	0.64	< 0.001	< 0.001	0.01	70.8	40.7	8.1	67
ΣHCH$_3$	0.55	< 0.001	< 0.001	0.02	97.5	49.2	5.37	192
α-HCH	0.55	< 0.001	< 0.001	0.56	91.5	47.5	0.343	192
β-HCH	0.55	< 0.001	< 0.001	0.01	93.1	49.1	7.13	190
ClBz	0.41	< 0.001	< 0.001	0.01	35.1	12.2	7.64	148
ΣPBDE$_{14}$	0.56	< 0.001	< 0.001	0.01	98.7	49.8	6.9	192
BDE-47	0.53	< 0.001	< 0.001	0.69	91.9	46.7	0.16	192
BDE-153	0.42	< 0.001	< 0.001	< 0.01	21.8	9.86	12.7	130
HBCDD	0.39	< 0.001	< 0.001	0.30	34.4	11.7	1.1	150

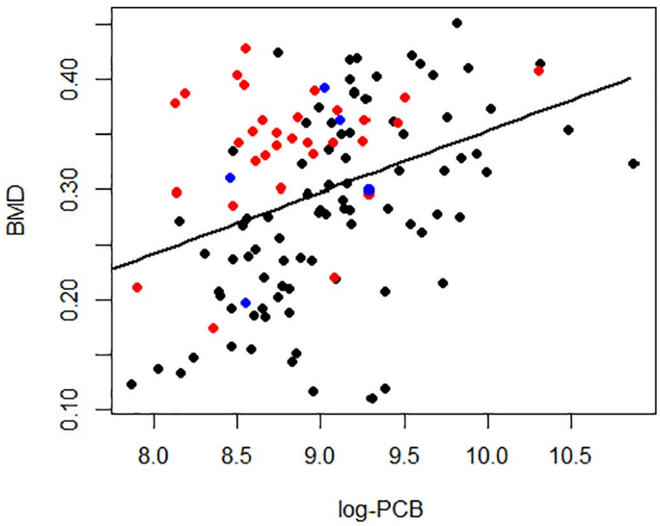

Fig. 4. Correlation between log-transformed concentrations of ΣPCB_{74} in the subcutaneous adipose tissue and baculum bone mineral density (BMD) values of East Greenland and Canadian polar bears collected in the 1999–2015 period (n = 192). Black: EG. Red: SH. Blue: WH. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. Considerations

Baculum density was significantly lowest in East Greenland polar bears despite the positive statistical correlations between BMD and POPs. The modelling of BMD vs. POPs using relative simple statistical models do not fully encompass the complex physiological homeostasis of baculum bone mineral density. Other important factors such as body mass, body condition and nutrients such as vitamins was not available. It is therefore difficult to pinpoint the exact physiological mechanisms that are involved in the control of baculum BMD and thereby fully understand how POP exposure may affect bone homeostasis. To study this further, knowledge of the bears' life history regarding seasonal variations, nutritional status and food availability is warranted (Desforges et al., 2016; Jenssen et al., 2015; Letcher et al., 2010; Sonne, 2010; Sonne et al., 2012). Logistic, ethical and economic issues make such investigations demanding. However, in order to clarify the mechanisms and shed further light on the complex relationship between POP exposure and baculum bone density, such investigations are needed (Sonne, 2010).

5. Conclusions

A geographical comparison showed that baculum BMD was significantly lowest in East Greenland polar bears when compared to Gulf of Boothia, Southern Hudson Bay and Western Hudson Bay subpopulations. Similar, a T-score calculation for EG suggested this subpopulation to be at risk of osteopenia when compared to WH bears as a reference group. Statistical analyses of individual baculum BMD and POP concentrations showed that BMD was positively correlated with several POP compounds including ΣPCB. Baculum density was significantly lowest in East Greenland polar bears despite the positive statistical correlations of BMD vs. POPs. Since on-going environmental changes are known to affect these, future studies need to incorporate nutritional, endocrine and genetic parameters to further understand how POP exposure may disrupt bone homeostasis and affect baculum BMD across polar bear subpopulations.

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Fig. 5. Example of a healed baculum fracture (encircled) in an adult polar bear from the low POP exposed Davis Strait subpopulation (specimen # L24857, Tag 1184). The baculum bone mineral density (BMD) was 0.41 g/cm^2 with a T-score of 1.35 meaning that this species is not in risk of having osteoporosis related fractures. Total length: 189 mm.

References

AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xii + 859 pp. Available at www.amap.no.

AMAP, 2004. Persistent Organic Pollutants in the Arctic. Amap Assessment 2002. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xvi + 310 pp. Available at www.amap.no.

AMAP, 2015. AMAP Assessment 2013: Human Health in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. Available at www.amap.no.

Atkinson, S.N., Stirling, I., Ramsay, M.A., 1996. Growth in early life and relative body size among adult polar bears (*Ursus maritimus*). J. Zool. 239, 225–234.

Barret, K., Brooks, H., Boitano, S., Barman, S., 2010. Ganongs Review of Medical Physiology, 23rd edition. (Chapter 23).

Bechshøft, T.Ø., Sonne, C., Jakobsen, J., Rigét, F.F., Born, E.W., Letcher, R.J., Jenssen, B.M., Dietz, R., 2016. Vitamins A and E in liver, kidney, and whole blood from organohalogen exposed East Greenland polar bears sampled 1994–2008: reference values and temporal trends. Polar Biol. 39, 743–754.

Daugaard-Petersen, R., Langebæk, R., Rigét, F.F., Letcher, R.J., Hydstrup, L., Bech Jensen, J.E., Bechshøft, T., Wiig, Ø., Jenssen, B.M., Pertoldi, C., Dietz, R., Sonne, C., 2018. Persistent organic pollutants, skull size and bone density of polar bears (Ursus maritimus) from East Greenland and Svalbard 1892–2015. Environ. Res. 162, 74–80.

Derocher, A.E., Stirling, I., 1998. Geographic variation in growth of polar bears (Ursus maritimus). J. Zool. 245, 65–72.

Derocher, A.E., Wiig, Ø., 2002. Postnatal growth in body length and mass of polar bears (*Ursus maritimus*) at Svalbard. J. Zool. 256, 343–349.

Desforges, J.P.W., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ. Int. 86, 126–139.

Dietz, R., Heide-Jørgensen, M.P., Härkönen, T., Teilmann, J., Valentin, N., 1991. Age determination of European harbour seal (*Phoca vitulina*). Sarsia 76, 17–21.

Dietz, R., Rigét, F.F., Sonne, C., Born, E.W., Bechshøft, T., McKinney, M.A., Letcher, R.J., 2013a. Part 1: three decades (1984-2010) of legacy contaminant trends in East Greenland polar bears (*Ursus maritimus*). Environ. Int. 59, 485–493.

Dietz, R., Rigét, F.F., Sonne, C., Born, E.W., Bechshøft, T.Ø., McKinney, M., Muir, D.C.G., Letcher, R.J., 2013b. Three decades (1983–2010) of contaminant trends in East Greenland polar bears (Ursus maritimus). Part 2: brominated flame retardants. Environ. Int. 59, 494–500.

Dietz, R., Gustavson, K., Sonne, C., Desforges, J.P., Rigét, F.F., McKinney, M.A., Letcher, R.J., 2015. Physiologically-based pharmacokinetic modelling of immune, reproductive and carcinogenic effects from contaminant exposure in polar bears (*Ursus maritimus*). Environ. Res. 140, 45–55.

Durner, G.M., Douglas, D.C., Nielson, R.M., Amstrup, S.C., McDonald, T.L., Stirling, I., Mauritzen, M., Born, E.W., Wiig, Ø., DeWeaver, E., Serreze, M.C., Belikov, S., Holland, M.M., Maslanik, J., Aars, J., Bailey, D.A., Derocher, A.E., 2009. Predicting 21st-century polar bear habitat distribution from global climate models. Ecol. Monogr. 79, 25–58.

Dyck, M., Bourgeois, J.M., Miller, E.H., 2004. Growth and variation in the bacula of polar bears (Ursus maritimus) in the Canadian Arctic. J. Zool. 264, 105–110.

Hensel, R.J., Sorensen, F.E., 1980. Age determination of live polar bears. Int. Conf. Bear. Res. Manag. 4, 93–100.

Herlin, M., Kalantari, F., Stern, N., Sand, S., Larsson, S., Viluksela, M., Tuomisto, J.T., Tuomisto, J., Tuukkanen, J., Jämsä, T., Lind, P.M., Håkansson, H., 2010. Quantitative characterization of changes in bone geometry, mineral density and biomechanical properties in two rat strains with different Ah-receptor structures after long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology 29, 1–11.

Jenssen, B.M., Dehli Villanger, G., Gabrielsen, K.M., Bytingsvik, J., Ciesielski, T.M., Sonne, C., Dietz, R., 2015. Anthropogenic flank attack on polar bears: interacting consequences of climate warming and pollutant exposure. Front. Ecol. 3, 1–7.

Johansson, S., Lind, P.M., Hakansson, H., Oxlund, H., Örberg, J., Melhus, H., 2002. Subclinical hypervitaminosis A causes fragile bones in rats. Bone 31, 685–689.

Kurtén, B., 1964. The evolution of the polar bear (*Ursus maritimus*). Acta Zool. Fenn. 108, 1–30.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043.

Letcher, R.J., Morris, A.D., Dyck, M., Sverko, E., Reiner, E., Blair, D.A.D., Chu, S.G., Shen, L., 2018. Legacy and new halogenated persistent organic pollutants in polar bears from a contamination hotspot in the Arctic, Hudson Bay Canada. Sci. Total Environ. 610-611, 121–136.

Lind, P.M., Larsson, S., Oxlund, H., Håkansson, H., Nyberg, K., Eklund, T., Örberg, J., 2000. Change of bone tissue composition and impaired bone strength in rats exposed to 3,3′, 4,4′, 5-pentachloro-biphenyl (PCB126). Toxicology 150, 43–53.

Lind, P.M., Bergman, A., Olsson, M., Örberg, J., 2003. Bone mineral density in male baltic grey seal (*Halichoerus grypus*). Ambio 32, 385–388.

Lind, P.M., Milnes, M.R., Lundberg, R., Bermudez, D., Örberg, J., Guilette, L.J., 2004. Abnormal bone composition in female juvenile american alligators from a pesticide-polluted lake (Lake Apopka, Florida). Environ. Health Perspect. 112, 359–362.

Manning, T.H., 1971. Geographical variation in the polar bears (*Ursus maritimus*). Can. Wildl. Serv. Rep. Ser. 13.

McKinney, M.A., Stirling, I., Lunn, N.J., Peacock, E., Letcher, R.J., 2010. The role of diet on long-term concentration and pattern trends of brominated and chlorinated contaminants in western Hudson Bay polar bears, 1991-2007. Sci. Total Environ. 408, 6210–6222.

McKinney, M.A., Letcher, R.J., Aars, J., Born, E.W., Branigan, M., Dietz, R., Evans, T.J., Gabrielsen, G.W., Peacock, E., Sonne, C., 2011. Flame retardants and legacy contaminants in polar bears from Alaska, Canada, East Greenland and Svalbard, 2005-2008. Environ. Int. 37, 365–374.

McKinney, M.A., Iverson, S.J., Fisk, A.T., Sonne, C., Rigét, F.F., Letcher, R.J., Arts, M.T., Born, E.W., Rosing-Asvid, A., Dietz, R., 2013. Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. Glob. Chang. Biol. 19, 2360–2372.

Molnár, P.K., Derocher, A.E., Klanjscek, T., Lewis, M.A., 2011. Predicting climate change impacts on polar bear litter size. Nat. Commun. 2, 186.

Norstrom, R.J., Belikov, S., Born, E.W., Garner, G.W., Malone, B., Olpienski, S., 1998. Chlorinated hydrocarbon contaminants in polar bears from eastern Russia, North America, Greenland and Svalbard: biomonitoring of Arctic pollution. Arch. Environ. Contam. Toxicol. 35, 354–367.

Paetkau, D., Amstrup, S.C., Born, E.W., Calvert, W., Derocher, A.E., Garner, G.W., Messier, F., Stirling, I., Taylor, M.K., Wiig, O., Strobeck, C., 1999. Genetic structure of the world's polar bear populations. Mol. Ecol. 8, 1571–1584.

R Development Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 3-900051-07-0. http://www.R-project.org/.

Sonne, C., 2010. Health effects from long-range transported contaminants in Arctic top predators: an integrated review based on studies of polar bears and relevant model species. Environ. Int. 36, 461–491.

Sonne, C., Dietz, R., Born, E.W., Rigét, F.F., Kirkegaard, M., Hyldstrup, L., Letcher, R.J., Muir, D.C.G., 2004. Is bone mineral composition disrupted by organochlorines in East Greenland polar bears (*Ursus maritimus*)? Environ. Health Perspect. 112, 1711–1716.

Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Rigét, F.F., Kirkegaard, M., Muir, D.C.G., 2006. Xenoendocrine pollutants may reduce size of sexual organs in east greenland polar bears (Ursus maritimus). Environ. Sci. Technol. 40, 5668–5674.

Sonne, C., Dietz, R., Born, E.W., Rigét, F.F., Leifsson, P.S., Bechshøft, T.Ø., Kirkegaard, M., 2007. Spatial and temporal variation in size of polar bear (Ursus maritimus) sexual organs and its use in pollution and climate change studies. Sci. Total Environ. 387, 237–246.

Sonne, C., Rigét, F.F., Jensen, J.E.B., Hyldstrup, L., Teilmann, J., Dietz, R., Kirkegaard, M., Andersen, S., Letcher, R.J., Jakobsen, J., 2008. Does the nutrition profile of vitamins, fatty acids and microelements counteract the negative impact from organohalogen pollutants on bone mineral density in Greenland sledge dogs (Canis familiaris)? Environ. Int. 34, 811–820.

Sonne, C., Wolkers, H., Rigét, F.F., Jensen, J.E., Teilmann, J., Jenssen, B.M., Fuglei, E., Ahlstrøm, Ø., Dietz, R., Muir, D.C., Jørgensen, E.H., 2009. Mineral density and biomechanical properties of bone tissue from male Arctic foxes (Vulpes lagopus) exposed to organochlorine contaminants and emaciation. Comp. Biochem. Physiol. C 149, 97–103.

Sonne, C., Letcher, R.J., Bechshøft, T.Ø., Rigét, F.F., Muir, D.C.G., Leifsson, P.S., Born, E.W., Hyldstrup, L., Basu, N., Kirkegaard, M., Dietz, R., 2012. Two decades of biomonitoring polar bear health in Greenland: a review. Acta Vet. Scand. 54, 15.

Sonne, C., Bechshøft, T.Ø., Rigét, F.F., Baagøe, H.J., Hedayat, A., Andersen, M., Bech-Jensen, J., Hyldstrup, L., Letcher, R.J., Dietz, R., 2013. Size and density of East Greenland polar bear (Ursus maritimus) skulls: valuable bio-indicators of environmental changes? Ecol. Indic. 34, 290–295.

Sonne, C., Dyck, M., Rigét, F.F., Beck Jensen, J., Hyldstrup, L., Letcher, R.J., Gustavson, K., Gilbert, M.T.P., Dietz, R., 2015. Penile density and globally used chemicals in Canadian and Greenland polar bears. Environ. Res. 137, 287–291.

Stirling, I., Derocher, A.E., 2012. Effects of climate warming on polar bears: a review of the evidence. Glob. Chang. Biol. 18, 2694–2706.

Stirling, I., Lunn, N., 1998. Environmental fluctuations in arctic marine ecosystem as reflected by variability in reproduction of polar bears and ringed seals. In: Woodin, S.J., Marquiss, M. (Eds.), Ecology of Arctic Environments. Blackwell Science, Oxford, pp. 167–181.

Verreault, J., Muir, D.C.G., Norstrom, R.J., Stirling, I., Fisk, A.T., Gabrielsen, G.W., Derocher, A.E., Evans, T., Dietz, R., Sonne, C., Sandala, G.M., Taylor, M.K., Nagy, J., Letcher, R.J., 2005. Chlorinated hydrocarbon contaminants and metabolites in polar bears (Ursus maritimus) from Alaska, Canada, East Greenland and Svalbard: 1996–2002. Sci. Total Environ. 351–352, 369–390.

WHO, 2007. WHO scientific group on the assessment of osteoporosis at primary health care level: summary meeting report. 2007 WHO Press, Geneva.

Wiig, Ø., Amstrup, S., Atwood, T., Laidre, K., Lunn, N., Obbard, M., Regehr, E., Thiemann, G., 2015. *Ursus maritimus*. The IUCN Red List of Threatened Species. pp. 2015. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T22823A14871490.en.

Wilson, D.E., 1976. Cranial variation in polar bears. In: Pelton, M.R., Lentfer, J.W., Folk, G.E. (Eds.), Bears – Their Ecology and Management: 447–453. Morges, Switzerland, IUCN.